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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.	CONFIRMATION NO.
10/708,204	02/16/2004	**Itzhak Bentwich**	050992.0201.03USCP	2203

37808 7590 12/15/2008

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EXAMINER	
WOLLENBERGER, LOUIS V	

ART UNIT	PAPER NUMBER
1635	

MAIL DATE	DELIVERY MODE
12/15/2008	PAPER

Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/708,204	BENTWICH, ITZHAK	
	Examiner	Art Unit	
	Louis Wollenberger	1635	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *30 September 2008*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *31,32 and 39-42* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *31,32 and 39-42* is/are rejected.

7)☐ Claim(s) _____ is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☐ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date _____.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status of Application/Amendment/Claims

Applicant's response filed 9/30/2008 has been considered. Rejections and/or objections not reiterated from the previous office action mailed 3/31/2008 are hereby withdrawn. The following rejections and/or objections are either newly applied or are reiterated and are the only rejections and/or objections presently applied to the instant application. The text of those sections of Title 35, U.S. Code not included in this action can be found in a prior Office action.

With entry of the amendment to the claims filed on 9/30/2008, claims 31, 32, and 39-42 are pending and under examination.

Applicant's amendment to the specification is acknowledged. The amendment has been entered into the application.

Priority

The previous Action acknowledged Applicant's claim for the benefit of a prior-filed application under 35 U.S.C. 119(e) or under 35 U.S.C. 120, 121, or 365(c), and 35 U.S.C. 119(a)-(d).

It was stated Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 119(a)-(d), 119(e), and 120 as follows: The later-filed application must be an application for a patent for an invention which is also disclosed in the prior application (the parent or original nonprovisional application or provisional application). The disclosure of the invention in the parent application and in the later-filed application must be sufficient to comply with the requirements of the first paragraph of 35

U.S.C. 112. See *Transco Products, Inc. v. Performance Contracting, Inc.*, 38 F.3d 551, 32 USPQ2d 1077 (Fed. Cir. 1994).

It was stated the disclosures of prior-filed Application Nos. 60/468,251, 10/649,653, 10/651,227, 10/707,147 11/24/2003, 10/604,985, 10/604,926, 10/604,727, 10/604,726, 10/707,975, 10/707,980, and PCT/IL03/00998 fail to provide adequate support or enablement in the manner provided by the first paragraph of 35 U.S.C. 112 for the instant claims drawn to SEQ ID NO:6527 and 15. That is, written description support for SEQ ID NO:6527 and 15 is not readily found in any of the prior filed applications to which priority is now claimed.

Response to Arguments

In reply, Applicant states instant SEQ ID NO:15 and 6527 are identical to SEQ ID NO:303 and 169, respectively, in Application No. 10/707,147, filed 11/24/2003. The Examiner agrees. Accordingly, in view of Applicant's Remarks, for purposes of this examination the earliest effective filing date of the instant claims is considered to be that of 10/707147: 11/24/2003.

The lack of priority finding is maintained, however, with regard each of the other prior-filed applications to which priority is claimed.

Specification

The specification is objected to because it refers to Tables 1-11 at paragraph 33 and elsewhere but the paper specification as filed does not contain any Tables labeled 1-11. The specification indicates that Tables 1-11 were supplied on CDs and are incorporated by reference

(paragraph 33). However, such incorporation by reference appears to be improper since it does not state with particularity what material therein is being relied on.

MPEP 608.01(p) indicates that in any application that is to issue as a U.S. patent, essential material may only be incorporated by reference to a U.S. patent or patent application publication. The Tables in question, and the essential information therein, are not a U.S. patent or a patent application publication. Note also that MPEP 608.01 (p) indicates that when incorporating material by reference "[p]articular attention should be directed to specific portions of the referenced document where the subject matter being incorporated may be found." In incorporating the tables by reference, Applicant makes no specific references to which portions of any of tables 1-11 in particular are relied on for support of the claimed subject matter.

The U.S. Court of Appeals, Federal Circuit, in *Zenon Environmental Inc. v. United States Filter Corp.*, 85 USPQ2d 1118 (Fed. Cir. 2007) stated that "To incorporate material by reference, the host document must identify with detailed particularity what material it incorporates and clearly indicate where that material is found in the various documents." (page 1124). With regard to the incorporation of tables 1-11 by reference, Applicant has not fulfilled these essential requirements.

The Examiner further notes the descriptive matter of an application should be limited to or at least pertinent to that which is claimed. A simple review of the matter in tables 1-11 (now in SCORE) shows that hundreds of thousands of unrelated sequences and several megabytes of information not remotely related to those now claimed is disclosed and incorporated by reference. Thus, the application contains a lengthy disclosure entirely outside the bounds of the claims.

Applicant is required to restrict the descriptive matter so as to be in harmony with the claims (MPEP § 1302.01) while avoiding new matter changes.

Claim Rejections - 35 USC § 101 and 112, First Paragraph

Claims 31, 32, and 39-42 remain rejected under 35 U.S.C. 101 because the claimed invention is not supported by a credible asserted utility.

Applicant asserts the specification teaches the instantly claimed isolated nucleic acids may be used to bind and regulate mRNA transcripts of SERPINA3 (see Remarks filed 2/4/2008, citing Table 7, lines 1488-1492). Indeed, a review of Table 7 (reproduced in part below) finds that instant SEQ ID NO:15 is asserted to regulate at least four different genes, including but not limited to SERPINA3. Evidently, according to the bioinformatic prediction program, SEQ ID NO:15 is capable of binding to and regulating multiple distinct genes.

```
GAM1032   CTAGACTGAAG CHAT     NM_020984.1  5  GCCTCAAGGGGTG   -   ---   AA          A      A
          CTCCTTGAGGA                        CGGCCCTCTCAG  CT AGA   CTG  GCTCCTTGAGG
                                                           GA TCT   GGC  TGGGGAACTCC
                                                             C   CCC   G-          G

GAM1032   CTAGACTGAAG CTSK     NM_000396.2  3  TCCTCAAGGTAGA C     TGAAG  -                A
          CTCCTTGAGGA                        AATGTCTAT      TAGAC     CT CCTTGAGGA
                                                           ATCTG     GA GGAACTCCT
                                                           T     TAAA-  T

GAM1032   CTAGACTGAAG MPO      NM_000250.1  5  CCCTCAAGGAGGT       TGAAG          A         A
          CTCCTTGAGGA                        CTGG          CTAGAC     CTCCTTGAGG
                                                           GGTCTG     GAGGAACTCC
                                                                 -----          C

GAM1032   CTAGACTGAAG MPO      NM_000250.1  5  CCTCAAGGAGGTC       TGAAG                    A
          CTCCTTGAGGA                        TGG           CTAGAC     CTCCTTGAGG
                                                           GGTCTG     GAGGAACTCC
                                                                 -----

GAM1032   CTAGACTGAAG SERPINA NM_001085.2  3  CCCATGGACTCTT           C-   T  A           A
          CTCCTTGAGGA 3                      CAGTCTGG      CTAGACTGAAG  TCC TG GG
                                                           GGTCTGACTTC  AGG AC CC
                                                                 TC   T  -

GAM1032   CTAGACTGAAG SERPINA NM_001085.2  3  GCCCATGGACTCT           C-   T  A  A         A
          CTCCTTGAGGA 3                      TCAGTCTGG     CTAGACTGAAG  TCC TG GG
                                                           GGTCTGACTTC  AGG AC CC
                                                                 TC   T  -  G

GAM1032   CTAGACTGAAG TNFRSF6 NM_152874.1  3  TCCTCAAGGACAT     AC   AGC                  A
          CTCCTTGAGGA                        TACTAG        CTAG  TGA   TCCTTGAGGA
                                                           GATC  ATT   AGGAACTCCT
                                                               --   AC-
```

While the asserted utility may be specific and substantial under the Utility Guidelines, the assertion is not credible for the following reasons.

In brief, the instant application claims bioinformatically predicted preprocessed and mature miRNA sequences corresponding to the 131-nt SEQ ID NO:6527 and the 22-nt SEQ ID NO:15, respectively. SEQ ID NO:15 is said by Applicant to bind and inhibit ChAT and SERPINA3 mRNA targets, based on bioinformatically predicted alignments, according to established rules governing miRNA target binding. Applicant asserts one of skill would more likely than not conclude the claimed nucleic acids may be used to modulate the expression of SERPINA3. Specific and substantial utility is thereby asserted based on bioinformatic data. The

asserted utility has not been experimentally verified. Indeed, there is no experimental evidence of even a single biological function. Function is asserted solely on the basis of a computer program designed to predict miRNA-like hairpin sequences and mature miRNAs derived therefrom by Dicer-catalyzed processing, which information is mined from raw genomic sequences.

At issue, then, is whether one of skill would more likely than not believe the nucleic acids predicted by Applicant's algorithm, such as the sequences now claimed, would have the specific and substantial utility predicted by the program.

1. In a previous response, Applicant submitted a Declaration under 37 CFR 1.132. Though made by a proclaimed expert in the art, and containing sound scientific reasoning, the Declaration represents nothing more than an opinion. While the declaration quantifies the effectiveness of other miRNA prediction algorithms, the declaration does not directly quantify the accuracy and/or false positive/false negative rate of the Inventor's algorithm, the program in question. Instead, the Declaration attempts to show the veracity of the instant prediction software by comparison to related prediction programs. Though unclear from the declaration, the assertion appears to be the instant algorithm is at least as effective as prior art algorithms. However, post-filing art (cited below) indicates it is difficult if not impossible to compare different algorithms without comparing their output using a common dataset, which does not appear to have been done here. The Declaration provides no experimental evidence validating either the predictive quality of the instant algorithm or the utility of the instantly claimed sequences.

Such evidence if collected in a statistically relevant manner would be indicative of the accuracy of the algorithm.

2. The Declaration similarly fails to address the utility of isolated nucleic acids complementary to either SEQ ID NO:6527 or 15. These sequences would clearly not be complementary to the target mRNA. The only perceived utility would be to either inhibit or detect the bioinformatically predicted miRNAs themselves. However, there is absolutely no evidence, beyond the algorithm, that the claimed miRNAs are biologically active in any manner, or even expressed by any cell. Thus, the complements to SEQ ID NO:6527 and 15 lack both substantial and credible utility since there is no evidence the targets of these complements have any utility or that any information of immediate, real-world value could be obtained from the use of sequences complementary to SEQ ID NO:6527 and 15.
3. The question remains whether the bioinformatically predicted miRNAs now claimed would, more likely than not, have the utility asserted. The answer lies in the predictive quality of the program used to identify the miRNAs and their target sites. A quantifiable value is not readily apparent to the Examiner from the facts of record. Indeed, the Examiner is unable to find any disclosure by the inventor either in the instant application or in the pre- or post-filing art clearly articulating the sensitivity or false positive rate of the instant algorithm. A simple statement supported by actual experimental evidence, showing the algorithm correctly predicts an miRNA and its activity more than half of the time and has an

acceptable false positive rate would be sufficient to overcome the instant rejection.

4. Currently, however, neither the Declaration nor the specification addresses this question directly or completely. In somewhat general terms the specification states at paragraph 275 that "assuming 80% accuracy of the HAIRPIN DETECTOR 114 and 80% accuracy of the DICER-CUT LOCATION DETECTOR 116 and 80% accuracy of the lab validation, this would result in 50% overall accuracy of the GAM oligonucleotide validated in the lab." Thus, it would seem the instant algorithm is correct about 50% of the time.
5. Further, it would appear from the teachings in the specification that multiple determinants govern the selection process.
6. Comparative algorithms used in the art are said to have false positive rates of between 22% and 39%. See Bentwich et al. (2005) *FEBS Lett.* 579:5904-5910, page 5907; and the Declaration, Point 4. See also Martin et al. (2007) *J. Biosci.* 32:1049-1052 at page 1049, 4th full paragraph.
7. Martin et al. (2007) *J. Biosci.* 32:1049-1052, reviewing the state of the art of miRNA prediction programs, state mammalian miRNA targets are considered difficult to predict because miRNA targets display only partial complementarity to the mature miRNA sequence (pg. 1049). Martin et al. further state that "Given the high level of both false-positives and false-negatives resulting from the application of current miRNA target prediction programs, it is clear that experimental testing of predicted miRNA targets is critically important in order to

validate/confirm any putative miRNA-target gene combination" (pg. 1050, 4th complete paragraph). Martin et al. further teach that miRNA prediction programs rely on sequence, structure, and evolutionary conservation information to predict genes likely to be targeted by miRNAs, but that the requirement for conserved sites means that non-conserved sites, which may represent real targets, are completely missed.

8. The post-filing art suggests that it is difficult to estimate the true false positive/negative rates of miRNA prediction programs because few validated miRNA targets are known. See Maziere et al. (2007) *Drug Discovery Today* 12:452-458, page 457. Maziere et al. in their article entitled "Prediction of miRNA Targets," further state that comparison of miRNA prediction efficiencies among different programs is not currently possible because many of the programs are not available for download and use on a common dataset; thus, Maziere et al. cast doubt on the reliability of the statements made in the Declaration, comparing similar programs to that used by the Inventor. Again, no evidence has been presented by Declarant directly comparing the output of the instant algorithm with the other cited programs when presented with a common dataset. Thus, there is no objective evidence to corroborate Declarant's opinion.
9. Smalheiser et al. (2006) *Methods Mol. Biol.* 342:115-127 in an article entitled "Complications in miRNA Target Prediction" state that complementarity between miRNAs and their targets is not the only factor that may govern which miRNA-mRNA target interactions are effective in vivo. One must consider the potential

importance of mRNA target secondary structure, as well as the strong possibility that RNA-binding proteins may participate in miRNA recognition. Furthermore, both miRNA and mRNA need to be coexpressed in proper amounts within the cell for effective interaction to occur, and A-to-I editing of RNA might abrogate potential mRNA targets from being effectively silenced by the RNA-induced silencing complex (page 124). Smalheiser et al. further teach that not all mammalian miRNAs interact with their targets via "short seeds," complementary regions of 6-8 nucleotides, but, instead, may interact via "long" seeds and perfect matches (page 115-6), and because new miRNAs are constantly being discovered this list of binding determinants may not be complete.

10. A search of putative target sites of the claimed miRNA, SEQ ID NO:15, using the miRanda program available at www.microrna.org, finds hundreds of putative target sites in hundreds of genes.
11. Thus, multiple factors are involved in miRNA-target binding and recognition.
12. In addition, the prior and post-filing art suggests that microRNA target specificity and function depends on the production of a dsRNA intermediate comprising the microRNA. See Cullen (2004) "Derivation and function of small interfering RNAs and microRNAs" *Viral Res.* 102:3-9, pages 4-6 and Fig. 1. This intermediate is required for effective incorporation of the miRNA guide strand into the RISC. Currently, there is no evidence the single stranded 22-mer, SEQ ID NO:15, as now claimed, which, if like most mature mRNAs, has at most 7 or 8 contiguous nucleotides complementary to the target gene, would regulate the

target gene on its own if transfected or endogenously expressed as a single stranded 22-mer in the cell.

13. While the 131-nt, partially self-complementary SEQ ID NO:6537 may reasonably be a substrate for dicer-catalyzed cleavage, and give rise to a dsRNA intermediate capable of inhibiting gene expression, there is no evidence to show a short dsRNA intermediate comprising SEQ ID NO:15 is a product derived from said cleavage.
14. Thus, in view of the totality of the evidence, one of skill would have reason to doubt the objective truth of the asserted utility. While the instant algorithm provides a list of putative miRNAs and corresponding target sites, there is reason to question whether the bioinformatic algorithm used to produce this list correctly identifies an miRNA and its function (i.e., at least one biological function) with minimally acceptable false positive and false negative rates such that one of skill would believe the miRNA would, more likely than not, inhibit the gene predicted by the software. Without experimental validation or any verifiable evidence of the accuracy and error rates of the instant program, and in view of the state of the art at the time of invention, one of skill would reasonably question the certainty of the prediction at the time of filing.
15. The skilled artisan would be led to believe only that the instantly claimed nucleic acids require further research to verify the asserted utility.

***

Claims 31, 32, and 39-42 also remain rejected under 35 U.S.C. 112, first paragraph, since the claimed invention is not supported by a credible asserted utility or a well established utility for the reasons set forth above, one skilled in the art clearly would not know how to use the claimed invention.

Response to Arguments

Applicant argues the Examiner has not presented evidence Applicant's algorithm would result in false positive identification of the claimed nucleic acid. Applicant argues one would expect miRNA bioinformation prediction programs of the type used in the instant application to be correct 61-78% of the time.

The Examiner respectfully disagrees. The Action cites evidence in the form of peer-reviewed journal articles, describing the state of the miRNA prediction art, suggesting significant false positive rates and recommending biological validation. The programs provide lists of candidate precursor and mature miRNAs, but do not replace experimental validation. As a practical matter, the Patent Office is not equipped to manufacture products by the myriad of processes put before it and then obtain prior art products and make physical comparisons therewith." *In re Brown*, 459 F.2d 531, 535, 173 USPQ 685, 688 (CCPA 1972).

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person

having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

The factual inquiries set forth in *Graham* **v.** *John Deere Co.*, 383 U.S. 1, 148 USPQ 459 (1966), that are applied for establishing a background for determining obviousness under 35 U.S.C. 103(a) are summarized as follows:

1. Determining the scope and contents of the prior art.
2. Ascertaining the differences between the prior art and the claims at issue.
3. Resolving the level of ordinary skill in the pertinent art.
4. Considering objective evidence present in the application indicating obviousness or nonobviousness.

Claims 31, 32, and 39-42 are rejected under 35 U.S.C. 103(a) as being unpatentable over Venter et al. (US Patent 6,812,339) and Zhao et al. (1997) GenBank Acc. No. AQ420078, first seen at NCBI on Mar 23 1999 12:30 AM, in view of

1. Buck et al. (Biotechniques (1999) 27(3): 526-538);
2. Hogan (US Pat. 5,541,308, July 30, 1996); and
3. Brown (1998) "In situ hybridization with riboprobes: An overview for veterinary pathologists" *Vet. Pathol.* 35:159-167.

Claim interpretation:

The claims read on DNA and RNA probes and primers and vectors thereof.

The rejection:

As shown by the alignment below, Venter et al. taught a nucleic acid sequence comprising instant SEQ ID NO:6527 and 15.

```
RESULT 1
US-09-949-016-13165
; Sequence 13165, Application US/09949016
; Patent No. 6812339
; GENERAL INFORMATION:
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;  APPLICANT: VENTER, J. Craig et al.
;  TITLE OF INVENTION: POLYMORPHISMS IN KNOWN GENES ASSOCIATED
;  TITLE OF INVENTION:  WITH HUMAN DISEASE, METHODS OF DETECTION AND USES THEREOF
;  FILE REFERENCE: CL001307
;  CURRENT APPLICATION NUMBER: US/09/949,016
;  CURRENT FILING DATE:  2000-04-14
;  PRIOR APPLICATION NUMBER: 60/241,755
;  PRIOR FILING DATE: 2000-10-20
;  PRIOR APPLICATION NUMBER: 60/237,768
;  PRIOR FILING DATE: 2000-10-03
;  PRIOR APPLICATION NUMBER: 60/231,498
;  PRIOR FILING DATE: 2000-09-08
;  NUMBER OF SEQ ID NOS: 207012
;  SOFTWARE: FastSEQ for Windows Version 4.0
; SEQ ID NO 13165
;   LENGTH: 346112
;   TYPE: DNA
;   ORGANISM: Human
;   FEATURE:
;   NAME/KEY: misc_feature
;   LOCATION: (1)...(346112)
;   OTHER INFORMATION: n = A,T,C or G
US-09-949-016-13165

  Query Match             100.0%;  Score 131;  DB 3;  Length 346112;
  Best Local Similarity   74.8%;  Pred. No. 6.3e-38;
  Matches   98;  Conservative   33;  Mismatches    0;  Indels    0;  Gaps    0;

Qy          1 GCUAGUCACUGGGGCAAAGAUGACUAAAACACUUUUCCUGCCCUCGAGGAGCUCACAGUC 60
              ||:||:|||:||||||||||:|||:|||||||::::||:||||:||||||||:|||||:|
Db     270570 GCTAGTCACTGGGGCAAAGATGACTAAAACACTTTTCCTGCCCTCGAGGAGCTCACAGTC 270629

Qy         61 UAGUAUGUCUCAUCCCCUACUAGACUGAAGCUCCUUGAGGACAGGGAUGGUCAUACUCAC 120
              :||:|:|:|:||:||||:||:|||||:|||::||||||||||||:||:||:||:|||
Db     270630 TAGTATGTCTCATCCCCTACTAGACTGAAGCTCCTTGAGGACAGGGATGGTCATACTCAC 270689

Qy        121 CUCGGUGUUGC 131
              |:|||:|::||
Db     270690 CTCGGTGTTGC 270700
```

As shown by the alignment below, Zhao et al. taught an isolated 684-nucleotide DNA sequence and BAC clone thereof comprising a sequence complementary to instant SEQ ID NO: 15

```
RESULT 1
AQ420078/c
LOCUS       AQ420078                684 bp    DNA     linear   GSS 23-MAR-1999
DEFINITION  RPCI-11-188K5.TV RPCI-11 Homo sapiens genomic clone RPCI-11-188K5,
            genomic survey sequence.
ACCESSION   AQ420078
VERSION     AQ420078.1  GI:4477802
KEYWORDS    GSS.
SOURCE      Homo sapiens (human)
  ORGANISM  Homo sapiens
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Primates; Haplorrhini;
            Catarrhini; Hominidae; Homo.
REFERENCE   1  (bases 1 to 684)
  AUTHORS   Zhao,S., Adams,M.D., Nierman,W., Malek,J., de Jong,P. and
            Venter,J.C.
  TITLE     Use of BAC End Sequences from Library RPCI-11 for Sequence-Ready
            Map Building
  JOURNAL   Unpublished (1997)
COMMENT     Other_GSSs: RPCI-11-188K5.TJ
            Contact: Shaying Zhao, William Nierman, Mark Adams
            Department of Eukaryotic Genomics
            The Institute for Genomic Research
            9712 Medical Center Dr., Rockville, MD 20850
            Tel: 301 838 0200
            Fax: 301 838 0208
            Email: hbe@tigr.org
            Clones are derived from the human BAC library RPCI-11. For BAC
```

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            library availability, please contact Pieter de Jong
            (pieter@dejong.med.buffalo.edu). Clones may be purchased from
            BACPAC Resources (http://bacpac.med.buffalo.edu/ordering) or from
            Research Genet cs (info@resgen.com). BAC end search page:
            http://www.tigr.org/tdb/humgen/bac_end_search/bac_end_search.html.
            Seq primer: T7
            Class: BAC ends.
FEATURES             Location/Qualifiers
     source          1. .684
                     /organism="Homo sapiens"
                     /mol_type="genomic DNA"
                     /db_xref="GDB:7572052"
                     /db_xref="taxon:9606"
                     /clone="RPCI-11-188K5"
                     /sex="Male"
                     /cell_type="Lymphocytes"
                     /clone_lib="RPCI-11"
                     /note="Vector: pBACe3.6; Site_1: EcoRI; Site_2: EcoRI;
                     RPCI11 Human Male BAC Library"
ORIGIN

  Query Match             95.0%;  Score 124.4;  DB 15;  Length 684;
  Best Local Similarity   73.8%;  Pred. No. 3e-30;
  Matches   93;  Conservative   32;  Mismatches    1;  Indels    0;  Gaps    0;

Qy          6 UCACUGGGGCAAAGAUGACUAAAACACUUUUCCUGCCCUCGAGGAGCUCACAGUCUAGUA 65
              :|||:||||||||||:|||:|||||||::::| :||||:||||||||:|||||:|:||:|
Db        684 TCACTGGGGCAAAGATGACTAAAACACTTTTCATGCCCTCGAGGAGCTCACAGTCTAGTA 625

Qy         66 UGUCUCAUCCCCUACUAGACUGAAGCUCCUUGAGGACAGGGAUGGUCAUACUCACCUCGG 125
              :|:|:||:||||:||:||||:|||||:||::||||||||||:||:||:||:||||:|||
Db        624 TGTCTCATCCCCTACTAGACTGAAGCTCCTTGAGGACAGGGATGGTCATACTCACCTCGG 565

Qy        126 UGUUGC 131
              :|::||
Db        564 TGTTGC 559
```

Venter et al. taught the use of probes and primers to detect and amplify the disclosed nucleic acids. It is said a probe or primer typically comprises a substantially purified oligonucleotide or oligonucleotide pair. The oligonucleotide typically comprises a region of nucleotide sequence that hybridizes under stringent conditions to at least about 12, 20, 25, 40, 50 or more consecutive nucleotides, and that the primer and probe sequences can readily be determined using the sequences disclosed.

While neither Venter et al. nor Zhao et al. expressly teach probes and primers comprising instant SEQ ID NO:6527 or 15 or their complements, in view of the disclosure of Buck et al., it would have been obvious to one of skill in the art at the time of invention that almost any complementary sequence of essentially any length suitable for detection of a nucleic acid could have been used to detect the sequences disclosed in Venter et al. and Zhao et al.: SEQ ID NO:13165 and GenBank Acc. No. AQ42007, respectively.

Buck analyzed the effect of primer design strategy on the performance of DNA sequencing primers. Specifically, Buck invited primer submissions from a number of labs (39) (page 532, column 3), with 69 different primers being submitted (see page 530, column 1). Buck also tested 95 primers spaced at 3 nucleotide intervals along the entire sequence at issue, thereby testing more than 1/3 of all possible 18 mer primers on the 300 base pair sequence (see page 530, column 1). When Buck tested each of the primers selected by the methods of the different labs, Buck found that every single primer worked (see page 533, column 1). Only one primer ever failed, No. 8, and that primer functioned when repeated. Further, every single control primer functioned as well (see page 533, column 1). Buck expressly states "The results of the empirical sequencing analysis were surprising in that nearly all of the primers yielded data of extremely high quality (page 535, column 2)." Therefore, Buck provides direct evidence that all primers would be expected to function, and in particular, all primers selected according to the ordinary criteria, however different, used by 39 different laboratories. It is particularly striking that all 95 control primers functioned, which represent 1/3 of all possible primers in the target region. This clearly shows that the selection and use of primers in primer extension methods yields predictable results.

Because primers and probes bind to their targets according to the same principles, it would be obvious to one of skill that each may be used according to the same purpose with the expectation each would bind the complementary target, whether via Northern blotting or in solution. In fact, one of skill would have even greater expectation of success given that probes need simply bind via Watson-Crick base-pairing and do not need to be extended as during PCR.

In view of the teachings of Buck, sequencing primers can be synthesized essentially anywhere along a given sequence of interest, and under optimal conditions they will reasonably be expected to perform adequately to yield sequence data. See page 533, left column, first full paragraph, and paragraph bridging pages 535 and 536. It would have been obvious to select a primer length of 22 nucleotides because those of ordinary skill normally use sequencing primers of 19-24 nucleotides in length (see Buck abstract). Accordingly, any 22 nucleotide fragment represented in either strand of the vector is considered to be obvious.

Further, the parameters and objectives for generating probes were well known in the art at the time the invention was made. For example, Hogan taught methods for generating target specific primers (col. 6-7, lines 50-67, lines 1-12), and provides extensive guidance for the selection of primers and probes. Hogan taught that "while oligonucleotide probes of different lengths and base composition may be used, oligonucleotide probes preferred in this invention are between about 15 and about 50 bases in length" (column 10).

Brown taught methods for making and using single stranded RNAs of virtually any length less than about 500 nt for detection of nucleic acids *in situ*. Brown also cites several other references pertinent to the riboprobe art.

Therefore it would have been prima facie obvious at the time of invention to make and use DNA and RNA probes and primers of essentially any length against essentially any region of the Venter et al. and Zhao et al. sequences for purposes of investigating the expression of said sequences as part of standard laboratory research with the anticipated success of detecting and or amplifying the corresponding sequences. It would further have been obvious to make and use

vectors encoding said primers as an easily accessible, economical, and renewable source of said probes and primers.

Response to arguments

In reply to the previous rejection, Applicant states Venter et al. do not teach preferred probes or primers corresponding to or reasonably suggesting the instant miRNA sequences or DNA equivalents.

This argument is not persuasive because Buck et al. and Hogan suggest making and using probes and primers to virtually any known sequence to detect and/or amplify said sequence for research purposes. The sequences disclosed by Venter et al. and Zhao et al., as part of the prior art, contained the instant sequences or sequences complemenary thereto and are of investigative interest to those of skill in the art, as it is the normal desire of scientists to investigate the functions of genes and gene products. Probes and PCR primers are routinely used in the art for these purposes. The instant claims are not limited to miRNAs. In fact the claims contain no functional language. One of skill would reasonably expect the set of probes and primers complementary to the target nucleic acid sequences would all be capable of hybridizing to said sequences in the manner necessary for use as probes and primers. While the set of sequences is large, it is finite and unambiguously described by the target sequences based on Watson-Crick base pairing rules.

Amending claims 31 and 32 to include functional language requiring the sequences inhibit the expression of a gene, would overcome the rejection as applied to claims 31, 32, 39, and 40. Nevertheless, this language must be crafted appropriately, since there is currently no disclosure teaching "the complement" of SEQ ID NO:6527 or 15 may be used to inhibit the

expression of a gene. Indeed, the complements may actually enhance the expression of the gene target by interfering with the miRNA.

Prior art made of record but not currently relied on

Lagos-Quintana et al. (2002) *Curr. Biol.* 12:735-739 taught a 22-nucleotide mouse miRNA, disclosed therein as miR-151, closely related and structurally similar to instant SEQ ID NO:15. See alignment below.

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RESULT 16
MMU459763
LOCUS       MMU459763                22 bp    mRNA    linear   ROD 05-JUL-2002
DEFINITION  Mus musculus microRNA miR-151.
ACCESSION   AJ459763
VERSION     AJ459763.1  GI:20799081
KEYWORDS    microRNA miR-151; miR-151 gene; miRNA.
SOURCE      Mus musculus (house mouse)
  ORGANISM  Mus musculus
            Eukaryota; Metazoa; Chordata; Craniata; Vertebrata; Euteleostomi;
            Mammalia; Eutheria; Euarchontoglires; Glires; Rodentia;
            Sciurognathi; Muroidea; Muridae; Murinae; Mus.
REFERENCE   1
  AUTHORS   Lagos-Quintana,M., Rauhut,R., Yalcin,A., Meyer,J., Lendeckel,W. and
            Tuschl,T.
  TITLE     Identification of tissue-specific microRNAs from mouse
  JOURNAL   Curr. Biol. 12 (9), 735-739 (2002)
   PUBMED   12007417
REFERENCE   2  (bases 1 to 22)
  AUTHORS   Tuschl,T.
  TITLE     Direct Submission
  JOURNAL   Submitted (06-MAY-2002) Dep. of Cellular Biochemistry, Max Planck
            Institute for Biophysical Chemistry, Am Fassberg 11, Goettingen
            37077, Germany
COMMENT     related sequence: TI88456669 (Trace Archive).
FEATURES             Location/Qualifiers
     source          1. .22
                     /organism="Mus musculus"
                     /mol_type="mRNA"
                     /db_xref="taxon:10090"
     gene            1. .22
                     /gene="miR-151"
     misc_RNA        1. .22
                     /gene="miR-151"
                     /product="microRNA miR-151"
                     /note="transcribed as larger precursor, predicted to form
                     hairpin"
ORIGIN

  Query Match             14.8%;  Score 19.4;  DB 6;  Length 22;
  Score over Length       88.2%;
  Best Local Similarity   71.4%;  Pred. No. 9.8e+04;
  Matches   15;  Conservative    5;  Mismatches    1;  Indels    0;  Gaps    0;

Qy         80 CUAGACUGAAGCUCCUUGAGG 100
              |:||||:|| ||:||::||||
Db          1 CTAGACTGAGGCTCCTTGAGG 21
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Kim et al. (2004) *PNAS* 101:360365 and Table 2, published online 12/22/03, taught the sequence of rat mir-151, which sequence is nearly identical to the complement of instant SEQ ID NO:15. See Table 2 (Supplementary material) therein.

Conclusion

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Louis Wollenberger whose telephone number is (571)272-8144. The examiner can normally be reached on M-F, 8 am to 4:30 pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, James (Doug) Schultz can be reached on (571)272-0763. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

Information regarding the status of an application may be obtained from the Patent Application Information Retrieval (PAIR) system. Status information for published applications may be obtained from either Private PAIR or Public PAIR. Status information for unpublished applications is available through Private PAIR only. For more information about the PAIR system, see http://pair-direct.uspto.gov. Should you have questions on access to the Private PAIR system, contact the Electronic Business Center (EBC) at 866-217-9197 (toll-free). If you would like assistance from a USPTO Customer Service Representative or access to the automated information system, call 800-786-9199 (IN USA OR CANADA) or 571-272-1000.

/Louis Wollenberger/
Examiner, Art Unit 1635
December 8, 2008